APPENDIX B

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: J. Brian Windsor, *et al.*	Prior Group Art Unit: 1651
Serial No.: 10/047,251	Examiner: Weber, Jon P.
Filed: January 14, 2002	Atty. Dkt. No.: TEXG:003USD1
For: GENETIC AND EPIGENETIC MANIPULATION OF ABC TRANSPORTERS AND ECTO-PHOSPHATASES FOR THE CONFERENCE OF DRUG RESISTANCE AND FOR THE LOSS OF DRUG RESISTANCE IN BIOLOGICAL SYSTEMS AND METHODS FOR THE DETECTION OF ECTO-PHOSPHATASE INHIBITORS	

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, J. Brian Windsor, declare that:

1. I have read U.S. Patent Application No. 10/047,251 filed on January 14, 2002 and am aware of the contents of said application.

2. I am a co-inventor of the present patent application, and am skilled in the areas of biochemistry and molecular biology.

3. Experimental results and evidence obtained in our laboratories are discussed as follows:

Enhancement of Herbicides with Ectophosphatase Inhibitors

Preliminary Greenhouse Herbicide Screen

Summary

A universal screen using several broadleaf and grass weed species was used to determine the potential for enhancement of approximately 25 active ingredients by one (or more) of 10 Ectophosphatase inhibitor lead compounds, identified by Formulas I (also labeled as Resistox 2, Res 2, or 2), II (Res 1 or 1), III (Res 19 or 19), VI (Res 4 or 4), IX (Res 8 or 8), XIV (Res 10 or 10), XVIII (Res 13 or 13), XV (Res 14 or 14), X (Res 15 or 15), and XII (Res 16 or 16) as set forth in the patent application. Results from greenhouse screening indicated that 50% of the active ingredients tested showed enhancement (overall performance or breadth of spectrum) when combined with a low rate of one or more Ectophosphatase inhibitor compounds.

Methodology

Test Species

Corn, cotton, and soybean and the following weeds were planted in a 10 inch pot:

Grasses:

Johnsongrass (SORHA), *Sorghum halepenese*

Small Crabgrass (DIGIS), *Digitaria ischaemum*

Barnyardgrass (ECHCG), *Echinochloa crusgalli*

Broadleaves:

Velvetleaf (ABUTH), *Abutilon theophrasti*

Ivyleaf Morningglory (IPOHE), *Ipomea hederacea*

Redroot Pigweed (AMARE), *Amaranthus sp*

About 10 seeds per weed species.

Assay

A primary screen with the crop and weed species listed above was used to identify which Ectophosphatase inhibitor compounds function as enhancers with an active ingredient. The primary screen involved applying herbicide and herbicide/Ectophosphatase inhibitor combinations to pots using a handheld sprayer. Both pre and postemergence applications were utilized, depending on label recommendations. Weeds were scored visually and compared to controls to determine efficacy of each treatment. A secondary screen, similar in methodology to the primary screen, was set up for confirmation of positive results from the primary screen.

Experimental Design

Pre or postemergence applications of herbicides were made per label directions. Applications were made containing herbicide only (with DMSO as a control) or herbicide with one of the Ectophosphatase inhibitor compounds. The herbicide (active ingredient) dosage was calculated from the label recommended rate, or the typical rate, for agricultural applications and was designated as the X rate. Three applications were made: 1/2X, 1/4X, and 1/8X. In all treatments, Ectophosphatase inhibitor was applied at a single rate of 0.02 lbs/A. DMSO was used as a solvent for all Ectophosphatase inhibitor compounds and in no case represented more than 0.1% of the volume applied in a treatment. All herbicide only treatments included DMSO at a concentration equivalent to herbicide/Ectophosphatase inhibitor treatments as a control. Plants

were grown in a mixed media using equal parts ProMix 'BX' and MetroMix 200. Temperature range was 20°C to 30°C. Pots were watered as needed using a handheld sprayer. Visual measurements and photos were taken after 4 weeks. No replicates were included in the primary screen.

Once target Ectophosphatase inhibitor compounds were identified in the primary screen a secondary screen was set up to confirm results from the primary screen using randomized duplicate treatments. Further information on increased breadth of spectrum was also obtained in the secondary screen. Weed spectrum in the secondary screen included many weed species utilized in the primary screen, as well as ones identified that may be "hard to control" weeds or weeds that are particular problems, based on label information and weed control ratings from university cooperators.

Results

The results from the herbicide screen indicate the efficacy of ectophosphatase inhibitors with herbicide active ingredients and products. The list here comprises specific ectophosphatase inhibitors and the products or active ingredients for which efficacy was demonstrated:

Formula II: flufenacet, metribuzin, amitrole, flucarbazone, trifluralin, rimsulsufuron, dicamba
Formula I: atrazine, isoxaflutole, bromoxynil, propanil, dicamba, sulfometuron methyl
Formula VI: flufenacet, metribuzin, flucarbazone, 2,4-D
Formula IX: flufenacet, metribuzin, amitrole, flucarbazone, cyhalofop, dicamba, sulfometuron methyl,
Formula XIV: atrazine, isoxaflutole, amitrole, fenoxaprop-p-ethyl
Formula XVIII: atrazine, flucarbazone, propanil, dicamba
Formula XV: atrazine, cyhalofop, 2,4-D
Formula X: trifluralin, s-metolachlor, trifloxysulfuron
Formula XII: flufenacet, metribuzin, foramsulfuron, flucarbazone, bromoxynil, cyhalofop, fenoxaprop-p-ethyl
Formula III: isoxaflutole, mesotrione, thifensulfuron

The following tables constitute selected results of the preliminary screen. Numbers below each weed species indicates level of control, with 0 meaning no control and 100 meaning total control (100% kill).

Resistox + Atrazine - preemergence

product	lb a.i./A	ECHCG	DIGIS	SORHA	IPOHE	AMARE	ABUTH
Atrazine	0.95	60	-	30	30	100	-
	0.48	30	10	20	65	100	-
	0.36	0	20	-	20	100	-
Atrazine	0.95 + 0,02	50	40	80		100	30
with Res 1	0.48 + 0,02	50	50	60	25	100	30
	0.36 + 0,02	20	10	30	0	100	10
Atrazine	0.95 + 0,02	85	85	10	100	95	100
with Res 2	0.48 + 0,02	30	50	50	0	100	10
	0.36 + 0,02	100	50	50	0	100	10
Atrazine	0.95 + 0,02	85	85	10	65	100	100
with Res 4	0.48 + 0,02	20	30	70	20	100	30
	0.36 + 0,02	30	30	60	0	100	20
Atrazine	0.95 + 0,02	60	50		75	100	85
with Res 8	0.48 + 0,02	40	30	20	50	100	0
	0.36 + 0,02	10	30		0	100	10
Atrazine	0.95 + 0,02	60	80	20	80	100	85
with Res 10	0.48 + 0,02	65	0	10	20	100	60
	0.36 + 0,02	20	35	10	0	100	15
Atrazine	0.95 + 0,02	90	75	80	100	100	100
with Res 13	0.48 + 0,02	40	30		20	100	35
	0.36 + 0,02	10	30	0	10	100	20
Atrazine	0.95 + 0,02	80	70	60	100	100	100
with Res 14	0.48 + 0,02	60	10		100	100	100
	0.36 + 0,02	0	5		20	100	20
Atrazine	0.95 + 0,02	50	60	80	100	100	100
with Res 15	0.48 + 0,02	50	60	60	50	100	
	0.36 + 0,02	30	35		20	100	
Atrazine	0.95 + 0,02	50	60	20	50	100	
with Res 16	0.48 + 0,02	50	60		45	100	
	0.36 + 0,02	10	10		15	100	
Atrazine	0.95 + 0,02	10	40	20	10	100	
with Res 19	0.48 + 0,02	30	40		100	100	
	0.36 + 0,02	10	10	0	0	100	

potential activator activity
potential lead compounds

Resistox + Buctril - postemergence

product	lb a.i./A		ECHCG	DIGIS	SORHA	IPOHE	AMARE	ABUTH
Buctril bromoxynil	0.25		10	0	10	100	100	-
	0.125		10	10	10	85	100	50
	0.0625		60	30	20	70	85	35
Buctril with Res 1	0.25	+ 0,02	20	10		100	90	
	0.125	+ 0,02	20	10		90	100	100
	0.0625	+ 0,02	10	10	0	60	100	
Buctril with Res 2	0.25	+ 0,02	90	90		100	100	100
	0.125	+ 0,02	30	20		95	100	100
	0.0625	+ 0,02	20	0		75	90	60
Buctril with Res 4	0.25	+ 0,02	10	10	10	80	100	100
	0.125	+ 0,02	20	10		70	100	50
	0.0625	+ 0,02	0	10		35	50	60
Buctril with Res 8	0.25	+ 0,02	30	10	20	90	100	100
	0.125	+ 0,02	20	10	10	70	100	100
	0.0625	+ 0,02	20	10		70	100	100
Buctril with Res 10	0.25	+ 0,02	30	10	30	85	100	100
	0.125	+ 0,02	25	10		70	100	100
	0.0625	+ 0,02	20	10		65	70	95
Buctril with Res 13	0.25	+ 0,02	40	20		90	100	100
	0.125	+ 0,02	30	20	20	70	100	100
	0.0625	+ 0,02	10	10		65	75	100
Buctril with Res 14	0.25	+ 0,02	20	30	30	75	100	90
	0.125	+ 0,02	30			85	100	80
	0.0625	+ 0,02	10	10	20	70	100	80
Buctril with Res 15	0.25	+ 0,02	40	10		100	100	100
	0.125	+ 0,02	30	20		85	100	100
	0.0625	+ 0,02	50	20		70	100	70
Buctril with Res 16	0.25	+ 0,02	65	10		90	100	100
	0.125	+ 0,02	25	0	10	70	100	90
	0.0625	+ 0,02	80	80		50	100	
Buctril with Res 19	0.25	+ 0,02	40	10	20	100	100	100
	0.125	+ 0,02	25	30	10	25	100	95
	0.0625	+ 0,02	30	20	10	70	100	100

potential activator activity
potential lead compounds

Resistox + Liberty - postemergence application

product	lb a.i./A	ECHCG	DIGIS	SORHA	SETVU	IPOHE	AMARE	ABUTH
		arnyard Gras	Crabgrass	ohnsongras	Green Foxta	Morningglor	Pigweed	Velvetleaf
Liberty	0.22	90	97.5	82.5	97.5	52.5	100	67.5
glufosina	0.11	50	97.5	65	100	80	100	62.5
Average	0.06	80	80	57.5	75	15	77.5	5
Liberty	0.22 + 0.0	97.5	100	100	100	100	100	100
with Res 1	0.11 + 0.0	62.5	100	85	100	60	87.5	97.5
Average	0.06 + 0.0	47.5	85	82.5	100	10	95	100
Liberty	0.22 + 0.0	97.5	100	90	100	60	100	100
with Res 2	0.11 + 0.0	77.5	95	95	100	77.5	100	97.5
Average	0.06 + 0.0	32.5	92.5	75	97.5	0	100	65
Liberty	0.22 + 0.0	100	100	95	100	100	100	100
with Res 4	0.11 + 0.0	80	95	85	100	35	100	60
Average	0.06 + 0.0	27.5	95	47.5	100	7.5	90	57.5
Liberty	0.22 + 0.0	90	100	95	100	97.5	100	95
with Res 8	0.11 + 0.0	27.5	100	77.5	100	52.5	100	80
Average	0.06 + 0.0	12.5	70	42.5	95	15	85	65
Liberty	0.22 + 0.0	90	100	97.5	100	77.5	100	100
with Res 10	0.11 + 0.0	35	92.5	55	100	37.5	90	92.5
Average	0.06 + 0.0	35	85	52.5	95	22.5	65	85
Liberty	0.22 + 0.0	95	97.5	95	100	87.5	100	97.5
with Res 13	0.11 + 0.0	42.5	87.5	50	100	55	100	55
Average	0.06 + 0.0	52.5	95	52.5	97.5	5	77.5	35
Liberty	0.22 + 0.0	72.5	97.5	70	100	87.5	100	97.5
with Res 14	0.11 + 0.0	52.5	95	90	100	47.5	100	72.5
Average	0.06 + 0.0	27.5	82.5	45	85	7.5	85	20
Liberty	0.22 + 0.0	85	95	82.5	97.5	67.5	100	97.5
with Res 15	0.11 + 0.0	70	100	72.5	100	65	85	95
Average	0.06 + 0.0	32.5	82.5	57.5	75	0	90	32.5
Liberty	0.22 + 0.0	90	97.5	92.5	100	65	100	90
with Res 16	0.11 + 0.0	50	77.5	75	100	47.5	90	87.5
Average	0.06 + 0.0	50	95	45	75	10	70	65
Liberty	0.22 + 0.0	85	100	95	100	60	100	75
with Res 19	0.11 + 0.0	72.5	90	70	92.5	25	100	62.5
Average	0.06 + 0.0	30	92.5	47.5	85	0	90	22.5

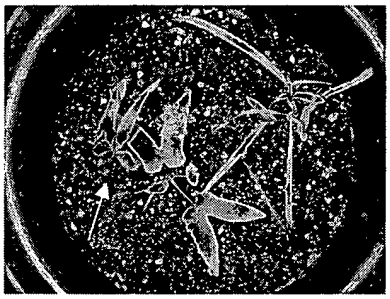
*Label rate is 0.44 lbs/A

Select Descriptions of Results

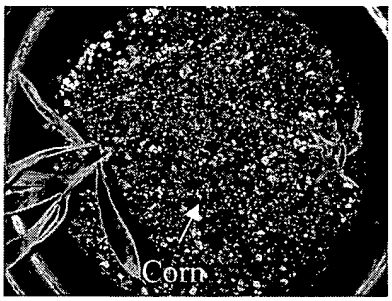
The results from further herbicide screening indicates the efficacy of ectophosphatase inhibitors with herbicide active ingredients and products. The list here comprises specific ectophosphatase inhibitors and the products or active ingredients for which efficacy was demonstrated.

Formula II: acetochlor (Surpass®), glufosinate (Liberty®)
Formula I: glufosinate (Liberty®)
Formula IX: paraquat

Surpass®
Multiple treatments confirm the ability of inhibitor to significantly improve the level of control of the pre-emergence herbicide Surpass (acetochlor) on "hard to kill" weed species. Results from secondary screening indicate that inhibitor compound I provides the greatest performance enhancement, as it allowed for significant control even at the lowest concentration of Surpass. With inhibitor I, the control of shattercane and yellow nutsedge did not dramatically decrease at lower concentrations of the herbicide, as noticed in herbicide-only treatments. Inhibitor I also afforded a high level of control of morningglory, a weed that was poorly controlled by the herbicide alone.



Surpass alone

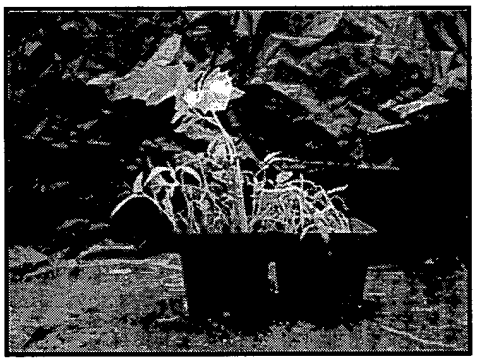


Surpass + inhibitor

Liberty®

Early screening against several weed species indicated the ability of the inhibitor technology to enhance the performance of Liberty (glufosinate). Results from primary and secondary screening efforts revealed that inhibitor compounds II and I afforded the greatest enhancement at reduced concentrations of Liberty. What was most significant was the impact of these compounds on "hard to control" weed species such as velvetleaf and pigweed. In further tests, the addition of inhibitor even allowed for control of the broadleaf species Jimsonweed, a weed not labeled for control by Liberty. The figure below reveals that Liberty alone provided no control of Jimsonweed; however the Liberty/inhibitor combination provided a high level of control. Results were confirmed by measurement of fresh and dry weights of plants post-treatment.

Liberty alone



Liberty + inhibitor

Paraquat

Primary and Secondary screening results confirmed the ability of inhibitor compound IX to significantly improve the level of control of the post-emergence herbicide active ingredient paraquat. Average results from secondary screening reveal that, as the concentration of paraquat is decreased, there is a dramatic drop in the level of control of morningglory and field bindweed. These 2 species are cited by the label as "hard to control." The addition of inhibitor IX allowed for equivalent control at the lower concentration.

Paraquat alone

Paraquat + inhibitor

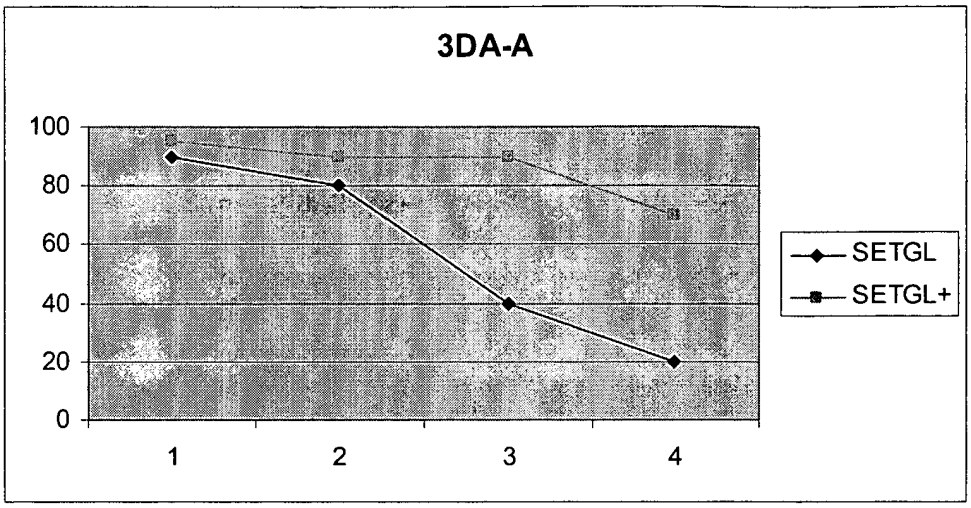
Further testing with paraquat and Ectophosphatase inhibitor compound IX demonstrated the ability of the inhibitor to allow for further increase in the level of control of paraquat against many species and also the ability of IX to allow for increased control of weeds at lower rates of paraquat, with rate 4 being the lowest rate of paraquat application. The following graphs represent selected results documented at 3 DAA and 14 DAA. Ratings were also taken at 7 DAA but are not included as they mirrored the results at 3 DAA. In each graph, the square data points represents application of paraquat with DMSO control only; the diamond data points represents application of paraquat with compound IX.

Paraquat rates

1.75	pt/a	1 (0.78 lbs)
0.875	pt/a	2 (0.39 lbs)
0.4375	pt/a	3 (0.19 lbs)
0.21875	pt/a	4 (0.10 lbs)

Rate of enhancer compound: 0.04 lbs/a

SETGL	=	*Setaria glauca* (Yellow foxtail)
SIDSP	=	*Sida spinosa* (Prickly Sida)
CONAR	=	*Convolvulus arvensis* (Field bindweed)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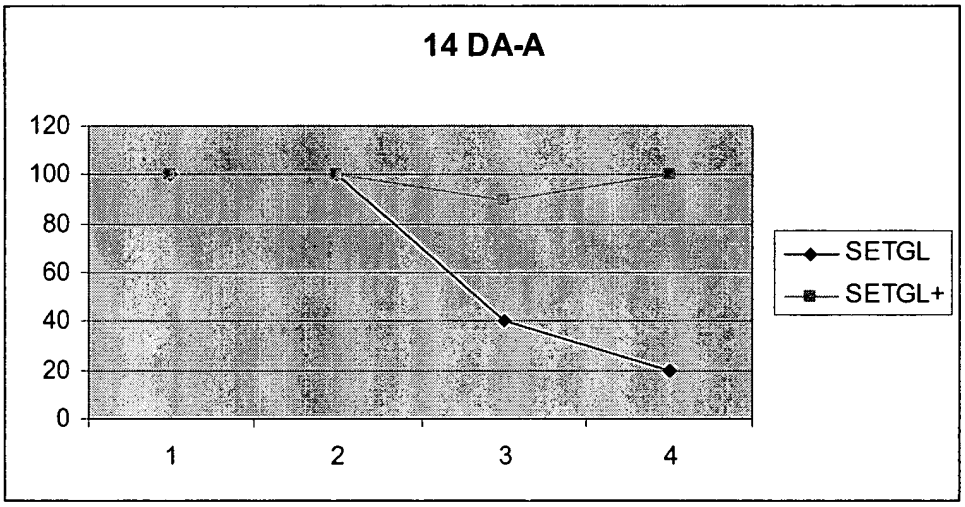
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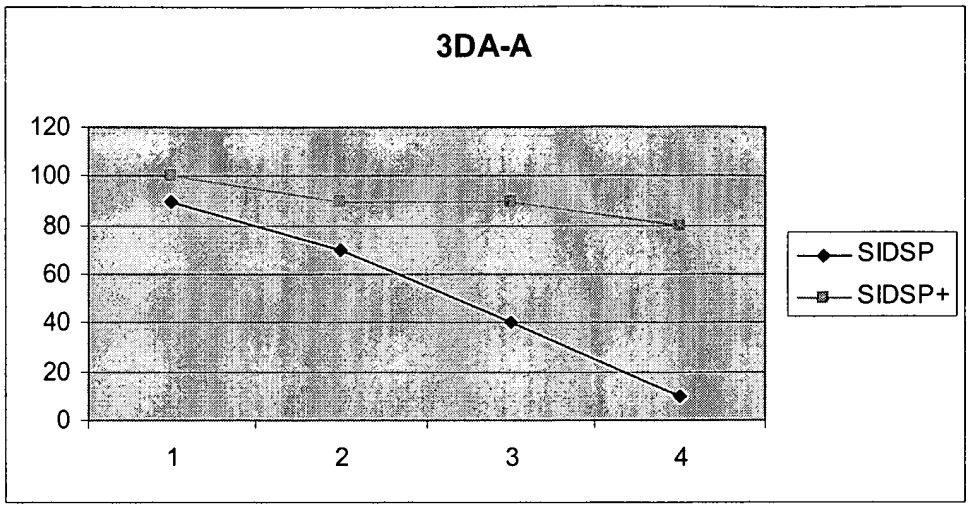
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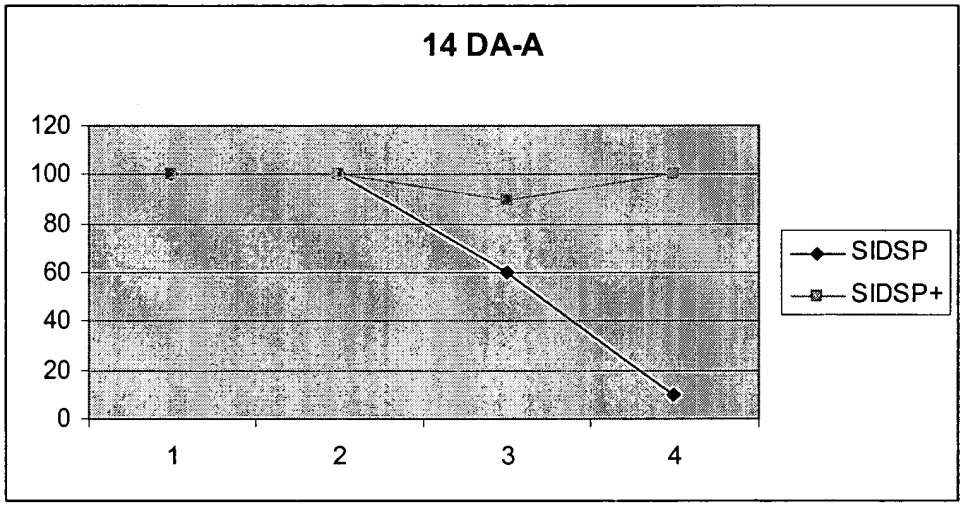
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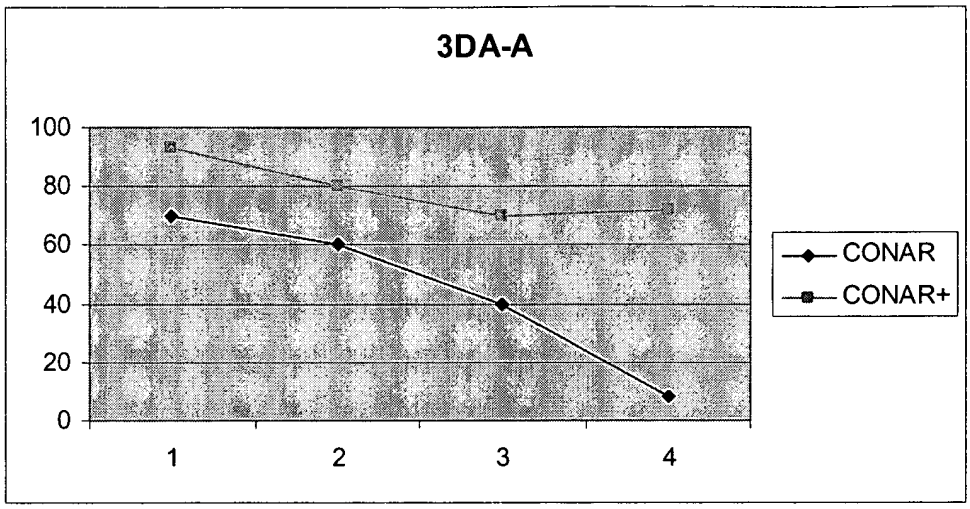
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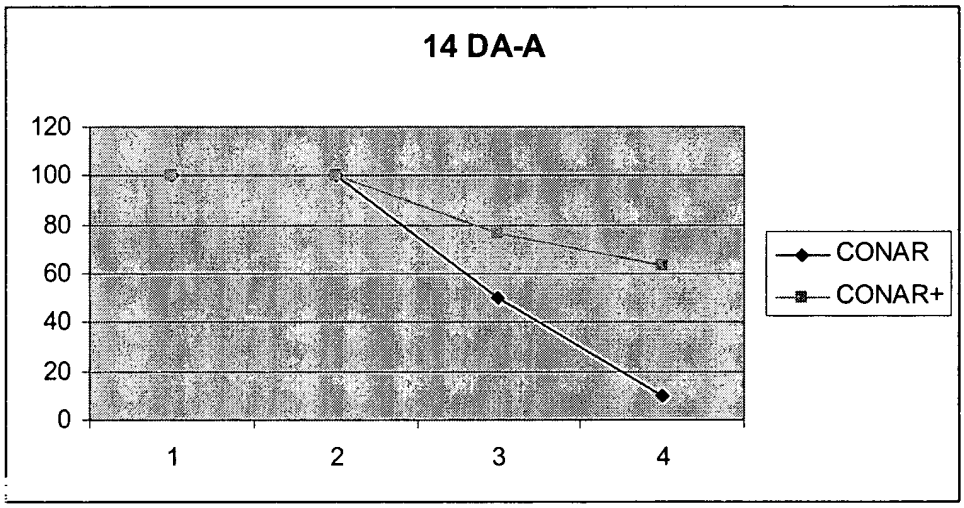
CONAR+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Preliminary Herbicide Field Test

The active ingredient atrazine (brand name Aatrex®) was selected for preliminary field trials in combination with ectophosphatase inhibitor Formula I. Below are selected results indicating that atrazine applied preemergence at 1 pound per acre with Formula I at 0.04 pounds per acre provides weed control similar to atrazine applied at 2 pounds per acre alone.

Atrazine with Res 2

Trial Information

Study Director: Prof. John Wilcut, N.C. State
Location: Lewiston, N.C.
Design: Randomized Complete Block
Crop: Corn DK 697
Reps: 3
Planting Date: April 23, 2003
Application Date: April 30, 2003
Rating Date: June 3, 2003

Weed code Crop code Rating type Rating unit		CROP STUNT %	STAND REDUC %	DIGSA CNTRL %	ELIEN CNTRL %	ABUTH CNTRL %	MOLVE CNTRL %	IPOLA CNTRL %	IPOHG CNTRL %
Trtmnt	lb A/A								
Aatrex	0.5	0	0	37	38	42	100	43	42
Aatrex	1.0	0	0	72	67	60	100	73	68
Aatrex	2.0	0	0	91	92	93	100	88	86
Aatrex Res 2	0.5 0.04	0	0	67	67	58	100	59	55
Aatrex Res 2	1.0 0.04	0	0	90	90	79	100	92	94
Aatrex Res 2	2.0 0.04	0	0	90	93	96	100	87	89

Weed code Crop code Rating type Rating unit		CROP STUNT %	STAND REDUC %	DIGSA CNTRL %	ELIEN CNTRL %	ABUTH CNTRL %	MOLVE CNTRL %	IPOLA CNTRL %	IPOHG CNTRL %
Trtmnt	lb A/A								
Aatrex	1.0	0	0	72	67	60	100	73	68
Aatrex Res 2	1.0 0.04	0	0	90	90	79	100	92	94

Weed code Crop code Rating type Rating unit		CROP STUNT %	STAND REDUC %	DIGSA CNTRL %	ELIEN CNTRL %	ABUTH CNTRL %	MOLVE CNTRL %	IPOLA CNTRL %	IPOHG CNTRL %
Trtmnt	lb A/A								
Aatrex	2.0	0	0	91	92	93	100	88	86
Aatrex Res 2	1.0 0.04	0	0	90	90	79	100	92	94

Weed code Crop code Rating type Rating unit		CROP STUNT %	STAND REDUC %	DIGSA CNTRL %	ELIEN CNTRL %	ABUTH CNTRL %	MOLVE CNTRL %	IPOLA CNTRL %	IPOHG CNTRL %
Trtmnt	lb A/A								
Aatrex	0.5	0	0	37	38	42	100	43	42
Aatrex Res 2	0.5 0.04	0	0	67	67	58	100	59	55

These experiments further demonstrate the efficacy of ecto-phosphatase inhibitors as set forth in the U.S. Patent Application Serial No. 10/047,251. Thus, the specification teaches one of skill in the art to use an ecto-phosphatase inhibitory compound to decrease drug resistance in a plant cell, without undue experimentation.

4. I hereby declare that all statements made herein on my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

_____ _____

Date J. Brian Windsor, Ph.D.